APPENDIX I
Enforcement Analytical Method

BUCKMAN INTERNATIONAL, INC.
MEMPHIS, TENNESSEE
OFFICIAL TESTING STANDARD

WSCP (Poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride]) by PVSAK Titration: Description of Method

SCOPE:

The method is suitable for analyzing WSCP product in the concentration range of 5 to 60 percent active ingredient. The method is applicable to the determination of WSCP in various products and formulations under proper conditions. Errors will result if other polyelectrolytes, either cationic or anionic, are present.

PRINCIPLE:

Under the conditions of the test, Poly(vinylsulfate, potassium salt) (PVSAK) reacts with WSCP and other cationic polyelectrolytes. When an excess of PVSAK is present in the sample solution the color of the indicator changes from blue to purple.

SAFETY:

This procedure does not claim to address all the possible safety and health aspects associated with its use.
It is the responsibility of the user to consult available information sources and to establish appropriate safety and health practices.

All chemicals used in this procedure must be handled in a manner consistent with the company Chemical Hygiene Plan and comply with OSHA guidelines governing Occupational Exposure to Hazardous Chemicals in Laboratories, 29 CFR Part 1910.

The Material Safety Data Sheets must be consulted for proper handling of the product, raw material and reagents. Always wear safety glasses. Wear rubber gloves, aprons, goggles, face shields or respirators when appropriate. Wash hands when appropriate.

Skin exposure to corrosive chemicals should be treated by washing the affected area for fifteen minutes with soap and water. Eye exposure should be treated by washing the affected area for fifteen minutes with cold running water. Obtain professional medical treatment when necessary or when in doubt about need.

In addition to the general safety requirements above, specific safety precautions for this procedure are:

1. **Nitric Acid** -- Oxidizer. Corrosive to skin, eyes and mucous and membranes. Contact with organic materials may cause fire. Harmful if inhaled. Wear protective clothing and goggles. Avoid contact.
2. **Silver Nitrate** -- Causes skin burns. Avoid contact.

APPARATUS:

NOTE: Equivalent apparatus can be used unless otherwise noted. The user can purchase from a preferred supplier for best price and service.

1. Analytical balance capable of weighing 0.0001 g, Mettler AE 200 (or equivalent).
2. Micro burette, 10 mL, Class "A", ASTM D-664, Kimble # 17027F-10.
3. Other laboratory glassware including beakers, pipettes and volumetric flasks.
4. pH meter capable of measuring millivolts, Corning model 125 (digital) (or equivalent).
5. Double junction reference electrode, Orion model # 900200 (or equivalent).
6. Silver billet indicating electrode, Beckman (or equivalent).

REAGENTS AND MATERIALS:

NOTE: Equivalent reagents and materials can be used unless otherwise noted. The user can purchase from a preferred supplier for best price and service.

1. Silver nitrate ($AgNO_3$), volumetric standard 0.1 N solution in water, Aldrich # 31,943-0, CAS Registry No. 7761-88-8.
2. Silver nitrate, volumetric standard 0.02 N; aliquot 100 mL of 0.1 N $AgNO_3$ to a 500 mL volumetric flask, dilute to the mark with deionized water and mix.
3. Hexadimethrine bromide (HDMBr)(Polybrene®), Aldrich # 10,768-9, CAS Registry 28728-55-4. [NOTE: HDMBr is hygroscopic.]
4. Hexadimethrine bromide solution, about 0.005 N; weigh about 0.5 g and dilute to 500 mL with deionized water.
5. Poly(vinylsulfate, potassium salt)(PVSAK), Kodak Cat. # 119 9686, CAS Registry No. 26837-42-3. Aldrich # 27,196-9, CAS Registry No. 26182-60-5. NOTE: the Kodak material dissolves more easily and is recommended if available.
6. Poly(vinylsulfate, potassium salt), about 0.0025 N; weigh about 0.5 g and add to 1000 mL of deionized water, stir until all of the soluble salt dissolves. Filter the solution to remove the insoluble material.

7. Toluidine Blue O, certified (Tolonium chloride), Aldrich #19,816-1, CAS Registry No. 92-31-9.
8. Toluidine Blue O, 0.1 % solution; Dissolve 0.1 g in 100 mL of deionized water.
9. Toluidine Blue O, 0.01 % solution; Aliquot 5 mL of 0.1 % Toluidine Blue solution and dilute to a volume of 50 mL.
10. Nitric acid, ACS reagent (69-71-%), Fisher # A200-212, CAS Registry No. 7697-37-2.
11. Nitric acid, 1 + 1 solution. Add 500 mL of ACS reagent Nitric acid to 500 mL of deionized water, mix and store in a glass stoppered bottle.
12. Deionized water, resistivity level of 1.0 megohm/cm or greater.

STANDARDIZATION OF SOLUTIONS:

1. Standardize HDMBr; Aliquot (in triplicate) 30 mL portions of HDMBr solution to an appropriate flask, dilute to about 50 mL with deionized water, and add 5 mL of 1+1 nitric acid.
2. Determine the millivolts e.m.f. at the end point as follows:
   Titrate a *trial aliquot* of HDMBr solution with 0.02 N $AgNO_3$ and record the e.m.f. and mL of titrant at 1 mL intervals. As the end point is approached, the e.m.f. changes will increase in magnitude. Make smaller additions (0.1 mL) of titrant and record the e.m.f. as this occurs. As the end point is passed, the e.m.f. changes become smaller again. Larger (1.0 mL) additions of titrate can again be made. Plot e.m.f. *vs.* mL of titrant. Note the e.m.f. where the slope reaches a maximum. Future titrations are made to this e.m.f.
3. Titrate each aliquot with 0.02 N silver nitrate. The silver nitrate can be added rapidly from the burette at first. As the end point is approached, each addition of titrant should be as small as possible. Record the volume of titrant needed to bring the e.m.f. to the value found in step 2.

4. $N_{HDMBr} = (N_{AgNO3} \times mL_{AgNO3}) / mL_{HDMBr}$

5. Standardize PVSAK; Aliquot (in triplicate) 4 mL portions of 0.005 N HDMBr solution to an appropriate flask, dilute to 50 mL with deionized water and add 2 mL of 0.01 % Toluidine Blue O solution.
6. Titrate with 0.0025 N (approx.) PVSAK solution until the indicator changes from blue to purple. Record the volume of titrant that causes the last color change.
7. Determine a blank against a solution of 50 mL of deionized water containing 2 mL of 0.01 % Toluidine Blue O solution. The blank is equal to mL's of PVSAK needed to give the purple end point.

8. $N_{PVSAK} = (N_{HDMBr} \times mL_{HDMBr}) / (mL_{PVSAK} - blank)$

PROCEDURE:

1. Prepare a sample solution by weighing accurately an appropriate sized sample. (For example: about 1.5 g of the WSKT-10 test material.

2. Transfer to a 500 mL volumetric flask, dilute to the mark with deionized water and mix.

3. Select an aliquot to give a titration of 6 to 9 mL (if using a 10 ml burette);(for example: a 5 mL aliquot for the the WSKT-10 sample mentioned in step 1 above.)

4. Dilute the aliquot to about 50 mL and add 2 mL of 0.01 % Toluidine Blue O solution.

5. Titrate with 0.0025 N PVSAK solution until the indicator changes from blue to purple. Record the volume of PVSAK that causes the last color change. [NOTE: A precipitate usually begins to form shortly before the end point is reached. Thus the end point usually goes from milky blue to milky purple.]

CALCULATION:

% WSCP = [(mL_{PVSAK} - blank) x N_{PVSAK} x 129.61 x 100] / [wt_{AN} x 1000]

[NOTE: Use the blank found in step 7 under Standardization of Solutions]
[NOTE: The equivalent weight of WSCP is 129.61]
[NOTE: wt_{AN} = sample wt x (aliquot / sample volume)]

REFERENCES:

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